

REMARKS

Applicants respectfully request reconsideration in view of the amendments to the specification. Early and favorable action is earnestly solicited.

With respect to Figures 7 and 8, applicants have removed all references to Figures 7 and 8 from the specification on pages 7-8, 92-93, and 97-99. Reconsideration and withdrawal of this objection is respectfully requested.

Regarding updating the priority information in the first line of the specification to include application 08/417,174, applicants have amended the first line of the specification to include U.S. Patent application 08/417,174 filed on April 5, 1995 now U.S. Patent No. 5,844,075 and to update the status of U.S. Patent application 08/231,565 filed on April 22, 1994 to reflect U.S. Patent No. 5,876,560. Applicants respectfully request reconsideration and withdrawal of this objection.

As requested, applicants have inserted SEQ ID NO: 26 as the SEQ ID NO related to Figure 4 of the Brief Description of the Drawings. Reconsideration and withdrawal of this objection is respectfully requested.

As required by 37 C.F.R. 1.121, "marked up" versions of the replacement paragraphs of the specification are attached hereto with additions indicated by underlining and deletions by brackets.

If any further questions arise, the Examiner is invited to call the undersigned at (212) 415-8564.

Favorable reconsideration in view of the herewith presented amendment and remarks is respectfully requested.

It is believed that all of the pending claims are in condition for allowance. Early and favorable action by the Examiner is earnestly solicited.

No additional fee is believed to be necessary.

The Commissioner is hereby authorized to charge any additional fees which may be required for this response, or credit any overpayment to Deposit Account No. 13-4500, Order No. 2026-4124US3. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE
IN THE SPECIFICATION

Please replace the first sentence of the first paragraph on page 1, lines 6-9 with the following:

This application is a continuation of United States Patent application 08/417,174 filed on April 5, 1995 now U.S. Patent No. 5,844,075, which is a continuation-in-part of United States Patent application 08/231,565 filed on April 22, 1994 now U.S. Patent No. 5,876,560, which is herein incorporated by reference in its entirety.

Please replace the paragraphs on page 7, lines 16-19 with the following:

Figure 4 show the nucleic acid sequence of the full length cDNA25 (SEQ ID NO: 26). The start and stop codons are underlined.

Figure 5A shows amino acid sequence of the full length cDNA25 (SEQ ID NO: 27). The antigenic peptide is underlined.

Please delete the paragraphs on page 7, line 31 through page 8, line 14 as indicated below.

[Figures 7A-7B show the location of gp100 epitopes and the DNA fragments tested for epitope analysis and recognition by CTL. Figure 7A. Five DNA fragments (D3, D5, D4, C4, 25TR) tested for epitope analysis are shown (---, identical amino acid). Locations of the identified epitopes are underlined. Figure 7B. Recognition by CTL (620-1, 620-2, 660-1, 1143, 1200) of COS 7 cells transfected with each DNA fragment in pcDNA3 plasmid along with HLA-A2.1 cDNA by IFN-* secretion assays are shown (+, recognized; -, not recognized).

Figures 8A-8D show titration of gp100 epitopes by sensitization of HLA-A2.1+ T2 cells for CTL lysis. Lysis of T2 cells preincubated with peptides was tested in a 4h ⁵¹Cr release cytotoxicity assay. Figure 8A, Lysis by TIL1200 of T2 cells incubated with G9₁₅₄(*) or G10₁₅₄(*). Figure 8B. Lysis by TIL620 of T2 cells incubated with G9₂₀₉(*) or G10₂₀₈(*). Figure 8C. Lysis by TIL660-1 of T2 cells incubated with G9₂₈₀(*). Figure 8D. Lysis by TIL660-2 of T2 cells incubated with G10-5(*).]

Please replace the paragraph on page 92, line 23 through page 93, line 22 with the following:

Peptides were synthesized by a solid phase method using a peptide synthesizer (model AMS 422; Gilson Co. Inc., Worthington, OH) (>90% purity). The peptides to be synthesized were selected from the reported human sequence of gp100 based on HLA-A2.1 binding motifs (Falk, K., (1991) *Nature* 351:290; Hunt, D. F., et al, (1992) *Science* 255:1261; Ruppert, J., et al., (1993) *Cell* 74:929; Kubo, RT, et al. (1994) *J Immunol.* 152:3913). The following peptides were tested: Eight 8-mer peptides (with residues starting at -199, 212, 218, 237, 266, 267, 268, 269[; see Figure 7A]), eighty-four 9-mer peptides with residues starting at - 2, 4, 11, 18, 154, 162, 169, 171, 178, 199, 205, 209, 216, 241, 248, 250, 255, 262, 266, 267, 268, 273, 278, 280, 273, 286, 287, 298, 290, 309, 316, 332, 335, 350, 354, 358, 361, 371, 373, 384, 389, 397, 399, 400, 402, 407, 408, 420, 423, 425, 446, 449, 450, 456, 463, 465, 485, 488, 501, 512, 536, 544, 563, 570, 571, 576, 577, 578, 583, 585, 590, 592, 595, 598, 599, 601, 602, 603, 604, 606, 607, 613, 619, 648[; see Figure 7A]) and seventy-seven, 10-mer peptides with residues starting at - 9, 17, 57, 87, 96, 154, 161, 169, 177, 197, 199, 200, 208, 216, 224, 232, 240, 243, 250, 266, 267, 268, 272, 285, 287, 289, 297, 318, 323, 331, 342, 350, 355, 357, 365, 380, 383, 388, 391, 395, 399, 400, 406, 407, 409, 415, 432, 449, 453, 457, 462, 476, 484, 489, 492, 511, 519, 536, 543, 544, 548, 568, 570, 571, 576, 577, 584, 590, 595, 598, 599, 601, 602, 603, 605, 611, 629[; see Figure 7A]) were synthesized. Possible epitopes identified in the first screening were further purified by HPLC on a C-4 column (VYDAC, Hesperia, CA) (>98% purity) and the molecular weights of the peptides were verified by mass spectrometry measurement as previously described (Example 3; Kawakami, Y., et al., (1994) *J.Exp.Med.* 180:347; Kawakami, Y., et al., (1994) *Proc Natl Acad Sci (USA)* 91:6458).

Please replace the paragraph on page 97, line 31 through page 99, line 5 with the following:

With the exception of G10-4, which required a concentration of 1ug/ml to sensitize T2 cells for CTL lysis (Example 3; Kawakami, Y., et al., (1994) *Proc Natl Acad Sci (USA)* 91:6458), all gp100 epitopes identified in this study could sensitize T2 cells for CTL lysis at a concentration of 1ng/ml[(Figures 8A-8D)]. G10-5 appeared to be inhibitory to the cytotoxic

activity of CTL at concentration greater than 10ng/ml since lysis of T2 cells incubated with G10-5 at more than 10ng/ml was repeatedly lower than at 1-10ng/ml in this assay condition in which the peptide was present in the medium during entire 4h cytotoxicity assay[(Figure 8D)]. The relative binding affinity of these epitopes to HLA-A2.1 was also measured using an in vitro competitive binding assay (Table 13). G9₁₅₄ had an higher binding affinity (50% inhibition of the standard peptide at 11nM) to the HLA-A2.1 molecule than G10₁₅₄ (1010nM) which contains an extra leucine at the C-terminus of G9₁₅₄, and could sensitize T2 cells at lower concentrations than G10₁₅₄[(Figure 8A)]. G9₂₀₉ also bound to HLA-A2.1 with higher affinity (84nM) than G10₂₀₈ (2080nM) which contains an extra threonine at the N-terminus, and could sensitize T2 cells at lower concentrations of peptide than G10₂₀₈[(Figure 8B)]. Thus, the 9-mer peptides were superior to the corresponding 10 mer peptides in the sensitization of T2 cells to CTL lysis, and they also had higher binding affinities to HLA-A2.1. This was also the case for the identified MART-1 9 and 10 amino acid peptides (M9-2, M10-3, M10-4) (Example 2; Kawakami, Y., et al., (1994). *J.Exp.Med.* 180:347). The results of the peptide titration in the T2 cell lysis assay correlated with the results of the HLA-A2.1 binding affinity as measured by the in vitro binding assay. The other gp100 epitopes, G9₂₈₀, G10-4, or G10-5 had binding affinities for HLA-A2.1 with 50% inhibition at 95nM, 483nM, or 13nM, respectively. The HLA-A2.1 binding affinities of the previously identified HLA-A2 restricted melanoma epitopes in MART-1 (Example 2; Kawakami, Y., et al., (1994) *J.Exp.Med.* 180:347) and tyrosinase (Wolfel, T., (1994) *Eur.J.Immunol.* 24:759) were also measured (M9-2(397nM), M10-3(2272nM), M10-4(5555nM), T9₁ (333nM), T9₃₆₉ (40nM)). Except for the 10mer peptides (G10₁₅₄, G10₂₀₈, GM10-3, GM10-4), for which overlapping 9-mer epitopes (G9₁₅₄, G9₂₀₉, M9-2) existed, all melanoma epitopes had either high (G9₁₅₄, G10-5, T9₃₆₉) or intermediate (G9₂₀₉, G9₂₈₀, G10-4, M9-2, T9₁) binding affinities to HLA-A2.1.